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Kinetics, Localization, and Mechanism of 5-Aminolevulinic Acid-Induced Porphyrin Accumulation in Normal and Barrett's-Like Rat Esophagus

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Background and Objectives: **Photodynamic therapy may selectively destroy Barrett's epithelium in the esophagus. To optimize photosensitizer administration, the kinetics of 5-aminolevulinic acid (ALA)-induced porphyrin accumulation in the normal and Barrett's-like esophagus were studied in the rat.**
Study Design/Materials and Methods: **Animals received 200 mg/kg ALA intravenously (n = 21) or orally (n = 21). Six rats served as controls. At t = 1, 2, 3, 4, 6, 12, and 24 hr, porphyrin concentration in the esophagus was measured by using chemical extraction, and porphyrin localization was determined by laser scanning microscopy (LSM). In addition, in 20 animals, porphobilinogen deaminase, ferrochelatase, and iron concentration were determined. In a second group (n = 24), an esophagojejunostomy was performed to induce a Barrett's-like esophagus. After 18 weeks, animals received ALA, and LSM was performed at t = 1, 2, 3, 4, 6, 8, and 12 hr.**
Results: **Porphyrin accumulation in normal mucosa was 3.5-fold higher than in muscularis, with a maximum at 3 hr after ALA administration. With LSM, strong homogeneous fluorescence of the squamous epithelium was shown, with minor fluorescence of submucosa and muscularis. In Barrett's-like epithelium, fluorescence was heterogeneous but was also restricted to epithelial cells. There was no difference in fluorescence intensity between Barrett's-like and adjacent squamous epithelium. Porphobilinogen deaminase activity was higher and iron concentration was lower in the mucosa than in the muscularis ($P < 0.001$).**
Conclusion: **ALA-induced porphyrin accumulation selectively occurs in esophageal mucosa, whether normal or Barrett's-like, compared with the muscularis, with a maximum at 3 hr after**

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ALA administration. Selectivity may be caused by a different activity of heme-synthetic enzymes or relative iron deficiency in the mucosa. Lasers Surg. Med. 24:3–13, 1999.

Key words: ferrochelatase; fluorescence; iron; photodynamic therapy; porphobilinogen deaminase

INTRODUCTION

Since 1970 the incidence of esophageal adenocarcinoma has been rising in Europe and in the United States at a rate greater than that of any other malignancy [1]. Barrett's epithelial metaplasia is a premalignant condition, probably arising from (duodeno)gastroesophageal reflux, that causes 30–125× greater risk of developing esophageal adenocarcinoma [2]. Antireflux medication such as proton-pump inhibitors, H2 receptor blockers, or eventually antireflux surgery are the current treatment options. Although reflux complaints disappear in almost 100% of treated cases, no effect on Barrett's epithelium itself has been shown, and malignant degeneration toward adenocarcinoma can still proceed [3]. Thus, most studies have advised using endoscopic surveillance for patients with Barrett's esophagus (BE). New forms of therapy are under investigation to completely eliminate Barrett's epithelium. Laser therapy is one of these new modalities. Local intraluminal laser coagulation, which causes thermal destruction and ablation of the lesion in combination with antireflux therapy, may lead to regeneration with squamous cell epithelium [4]. A disadvantage is the relative uncontrolled damage through the entire wall, which often leads to strictures and perforations. Regeneration with both squamous and columnar epithelia has also been described [5].

In photodynamic therapy (PDT), laser light is used to activate a photosensitizing drug previously administered to accumulate in (pre)malignant tissues. This results in a photochemical reaction, with the generation of singlet oxygen causing cell death. The best known clinical photosensitizers are hematoporphyrin derivate (HpD) and its more purified form, Photofrin®. Although clinical studies have shown some effectiveness in the treatment of BE and superficial esophageal cancer, they have three main drawbacks [6,7]. They cause prolonged skin sensitivity to light, thus requiring avoidance of sunlight, and offer limited tumor selectivity [8–10]. Moreover, strictures requiring dilatation are a frequent complication when using HpD or Photofrin® [6,7]. A new method of photosensitization is the administration of the natural porphyrin precursor 5-aminolevulinic acid (ALA) [11]. The synthesis of ALA is rate limiting in the heme-synthetic pathway [12]. The administration of exogenous ALA bypasses both the rate-limiting step and the feedback control in the heme biosynthesis and leads to accumulation of protoporphyrin IX (PpIX) in certain types of cells and tissues [13]. ALA-PDT could therefore be a suitable therapy for BE. Although some clinical case reports have described promising results, remaining islands of columnar epithelium after therapy have been found in several studies [14–17]. These therapy-resistant cells may be eliminated by adjusting the ALA-PDT treatment.

The present study was carried out to determine the optimal timing of ALA administration with respect to subsequent therapeutic illumination. In the first experiment, the kinetics and localization of ALA-induced porphyrin accumulation in the rat normal esophagus were studied. To understand the mechanism of porphyrin accumulation, ALA and iron concentrations and activity of porphobilinogen deaminase (PBGD) and ferrochelatase were determined in the different layers of the esophagus. In the second experiment, with laser scanning microscopy, localization and relative concentration of ALA-induced porphyrin accumulation in Barrett's-like and adjacent normal esophagus were studied in an animal model.

MATERIALS AND METHODS

Animals and Materials

The experimental protocol was approved by the Committee on Animal Research of the Erasmus University. Ninety-two male adult Wag/Rij rats (Harlan CPB, Austerlitz, The Netherlands), weighing 180–200 g were used for the experiments. They had free access to rat chow and tap water. ALA was obtained from Sigma Chemical Company (Zwijndrecht, The Netherlands) with a purity of 98%. The following reagents were purchased from Porphyrin Products (Logan, UT): PpIX, disodium salt, and zinc PpIX. Tris-HCl was

purchased from Boehringer Mannheim (Mannheim, Germany), and other chemicals were purchased from Merck (Darmstadt, Germany).

Experimental Design

Forty-eight animals were randomly assigned to two study groups of 21 animals each and one control group of six animals. In the study groups, the animals were administered 200 mg/kg ALA dissolved in phosphate buffered saline (PBS) either by intravenous injection (i.v.) into the penile vein under ether anesthesia (n = 21) or by oral gavage (p.o.; n = 21). Control animals received PBS either i.v. (n = 3) or p.o. (n = 3). In the study groups, after administration of ALA, the animals were placed in subdued light. They were killed at 1, 2, 3, 4, 6, 12, and 24 hr after administration in groups of three. Control animals were killed at 4 hr. In subdued light, a small piece of the distal esophagus was excised and immediately snap frozen and stored at −80°C for fluorescence microscopic study. The rest of the esophagus was then resected and opened longitudinally. The mucosa was surgically separated from the underlying muscularis. The separated tissues were frozen immediately and stored at −80°C until chemical extraction.

Twenty additional untreated rats were used to determine ferrochelatase and PBGD activity (n = 10) and iron concentration (n = 10) in the esophageal mucosa and underlying muscular layer.

Barrett's-like Esophagus

Twenty-four animals underwent an esophagojejunostomy with total gastrectomy according to the model of Levrat et al. [18] to induce duodenoesophageal reflux and subsequent Barrett's-like columnar epithelium [18,19]. A median laparotomy was performed, and the esophagus was dissected 2 mm above its entry in the stomach. The stomach was resected and the duodenum was closed. Thereafter, the esophagus was anastomosized end-to-side to the jejunum, approximately 5 cm distal to the Treitz ligament. After 18 weeks of induced duodenal reflux, 21 rats received i.v. 200 mg/kg ALA. Rats were killed at 1, 2, 3, 4, 6, 8, and 12 hr after ALA administration (n = 3/time point). Three controls received PBS i.v. and were killed 3 hr later. The esophagus was dissected and cut into seven 5-mm pieces; the most distal piece was approximately 2 mm proximal to the anastomosis. The samples were immediately snap frozen and stored at −80°C for fluorescence microscopic study.

Determination of ALA Concentration

ALA was extracted from tissue samples with a mixture of dimethylsulfoxide (DMSO) and methanol (MeOH; 30/70 v/v) and quantified by using a fluorimetric enzyme assay [20,21]. In brief, tissue homogenates were mixed with 480 μl DMSO/MeOH and centrifugated; 200 μl of ALA standard solution (0.1–2 nmol/ml) or diluted tissue homogenate were mixed with 50 μl of an enzyme mixture of PBGD and ALA-D in Tris-HCl buffer or with 50 μl of Tris-HCl buffer for blank detection. The samples were incubated overnight, and the reaction was stopped by adding 750 μl stop buffer (one part Tris-HCl and two parts trichloroacetic acid). The samples were then placed on an ultraviolet (UV) light source (350 nm Woods light) to convert porphyrinogens into porphyrins and centrifugated. The porphyrin concentration in the supernatant was determined in a Perkin Elmer fluorimeter (LS 5B; Nieuwerkerk a/d IJssel, The Netherlands) by using a red-sensitive photomultiplier. The ALA standard line was used to calculate the original ALA concentration in tissue concentration (nmol/mg protein).

Porphyrin Analysis

The analysis was carried out according to Chisolm and Brown [22], with some modifications [20,22]. To tissue homogenates, HCL and ethylacetate/glacial acid were added. After centrifugation, the ethyl-acetate phase with some protein is removed, and after a second centrifugation the HCL phase is measured in a fluorescence spectrometer LS 5B with a red-sensitive photomultiplier (Perkin Elmer), at an excitation wavelength of 410 nm and an emission wavelength of 650 nm. Emission at 650 nm in a direct extraction assay as described above was found to be proportional (⩾90%) to PpIX quantification as determined by a limited number of high performance liquid chromatography (HPLC) porphyrin seperations. Porphyrin standards were analyzed separately for actual concentration by using UV spectroscopy and the molar extinction coefficient (ϵ_{407} = 0.275 $l \cdot mol^{-1} \cdot cm^{-1}$). Recovery of porphyrins was checked by adding standard PpIX to the samples. A recovery between 90 and 100 was achieved.

Protein was measured by using the method of Lowry et al. [23].

Fluorescence Microscopy

This method has been discussed in detail elsewhere [20]. In brief, in subdued light, tissue blocks were cut into sections of 10 μm thick (four sections per sample) and stored at –80°C. Just prior to fluorescence microscopy, the sections were thawed. Preparations were imaged by using a helium/neon laser (633 nm) for fluorescence excitation. After passing a 665 longpass filter, the emitted light was detected by a photomultiplier. All images were recorded in one session to avoid errors due to daily differences in microscope settings. The digital images were analyzed with the Kontron KS-400 image analysis program (Kontron Electronik, Munich, Germany). The respective areas of interest were outlined manually or determined by interactive thresholding. After that, the mean fluorescence (integrated fluorescence/surface area) in each area was automatically calculated. After fluorescence microscopy, sections were fixed in formalin, sectioned, and stained with hematoxylin and eosin (H&E) for comparative histological analysis.

Determination of PPGD and Ferrochelatase Activity

The samples were homogenized in sterile water (1:10 w/w) by using a Potter Elvehjem homogenizer. PBGD was measured as described by Wilson et al. [24] after an initial incubation at 55°C for 60 min and cooling to room temperature to destroy the activity of uroporphyrinogen decarboxylase and to prevent further metabolism of uroporphyrinogen. Ferrochelatase was measured by a modification of the method of Li et al. [25]. Fifty microliters of the homogenate were added to 100 μl 0.25 mol/l Tris-HCl buffer, pH 8.2, containing 8 g/l Triton X-100 and 1.75 mmol/l palmitic acid. Fifty microliters of a 250-μmol/l solution of PpIX in KOH 0.01 mol/l were added, and the reaction was started by the addition of 50 μl of a 200-μmol/l solution of zinc acetate in water. The mixture was incubated at 37°C for 60 min, and the reaction was stopped by the addition of 1 ml of dimethylsulfoxide:methanol (30:70). After subsequent centrifugation in an Eppendorf centrifuge for 5 min at 1,800*g,* 100 μl of supernatant were injected on a Perkin Elmer HPLC with a reversed-phase Chrompack RP18 column, with acetone/methanol/water/formic acid (560:240:200:2) 1 ml/min as a mobile phase. Zinc PpIX was detected by a Perkin Elmer LS40 fluorometer, with an excitation wavelength of 415 nm and an emission wavelength of 580 nm. Results were expressed as nanomoles per milligram of protein.

Determination of Iron Concentration

The concentration of iron was measured spectrophotometrically by using a modification of the method described by Lauber, with a wavelength of 562 nm [26].

Statistical Analysis

The values are expressed as means ± standard error of the mean (SEM). Comparisons were made using Student's t test. Total accumulation of porphyrins after oral and intravenous ALA administration was calculated by using an area under the curve analysis. A value of $P < 0.05$ was considered significant.

RESULTS

ALA Distribution

Maximum ALA concentration in mucosa and muscularis was reached at 1 hr after p.o. and i.v. ALA administration. In the p.o. group, peak ALA concentration was higher in mucosal tissue than in muscularis (2.08 ± 0.24 vs. 1.11 ± 0.18 nmol/mg protein, $P = 0.03$); in the i.v. group this was not statistically significantly different (1.46 ± 0.22 vs. 1.26 ± 0.30 nmol/mg protein, $P = 0.06$; Fig. 1). There was no difference in maximum ALA concentration in both mucosa and muscularis after p.o. or i.v. administration. After 1 hr, in both groups and tissue layers, ALA concentration declined sharply, with a half-life of approximately 1 hr, and was at background levels at 4 hr after ALA administration.

Porphyrin Distribution

Basal porphyrin concentration was higher in the mucosa than in the muscularis of the squamous-lined esophagus ($P = 0.049$; Fig. 1). Peak porphyrin levels, reached at 3 hr after ALA administration, were higher in mucosa (90.1 ± 10.0 pmol/mg protein in the p.o. group and 86.8 ± 12.8 pmol/mg protein in the i.v. group) than in muscularis (26.7 ± 3 .1 and 32.6 ± 3.4 pmol/mg protein; $P = 0.003$, p.o. and $P = 0.015$, i.v.). A maximal mucosa:muscularis ratio of approximately 3.5 was found at 2 hr after ALA administration in both groups. Total accumulation of porphyrins (area under the curve) was also higher in the mucosa than in the muscularis and was equal in the p.o. and i.v. groups. At 6, 12, and 24 hr after receiving

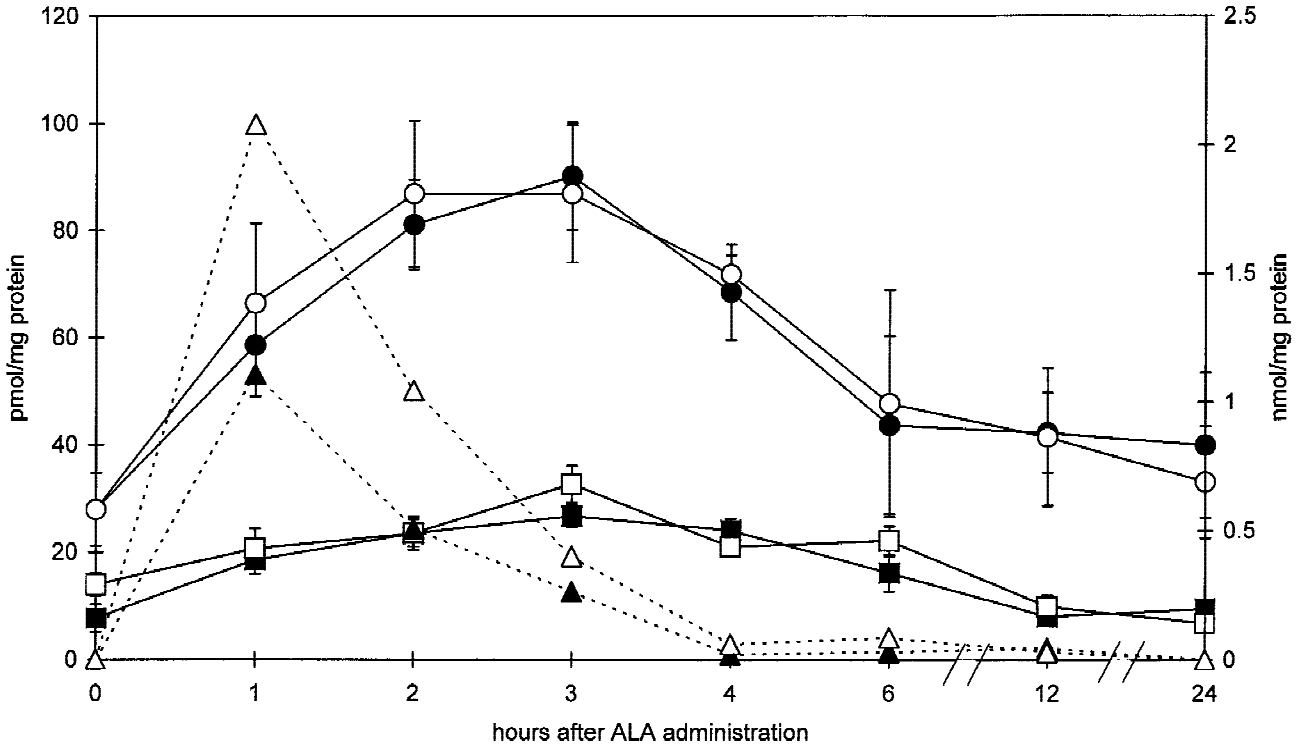

Fig. 1. Extracted porphyrin levels (solid lines, left y axis) and tissue 5-aminolevulinic acid (ALA) concentration (dashed lines, right y axis) after administration of 200 mg/kg ALA. Each point indicates the mean level of three rats ± SEM. The ALA and porphyrin levels of control rats are given at 0 hr. Open circles, mucosa, intravenous ALA; solid circles, mucosa, oral gavage ALA; open squares, muscularis, intravenous ALA; solid squares, muscularis, oral gavage ALA; open triangles, ALA concentration in esophageal mucosa; solid triangles, ALA concentration in esophageal muscularis.

ALA, porphyrin levels had returned to those levels found in control animals.

Fluorescence Imaging

Areas with strong autofluorescence were found in control animals (Fig. 2). Comparison with the H&E stainings showed that this fluorescence was restricted to the luminal surface of the esophageal mucosa with nests of food (e.g., chlorophyll and bacteria). In all sections, these autofluorescent areas were present. After administration of ALA, a strong, broad, homogeneous fluorescence of the basal cell layer of the squamous epithelium was found (Fig. 3A,B). Weak fluorescence was apparent in the muscularis mucosae. The keratin layer, lamina propria of the mucosa, the submucosa, and the muscularis showed fluorescence near background level. The fluorescence intensity of the different esophageal layers after analysis of the digital images are shown in Figure 4. Fluorescence level of the basal cell layer increased rapidly, reaching a maximum at 3 hr after ALA administration. Thereafter, fluorescence decreased to background levels at 24 hr. Fluorescence of the muscularis mucosae showed the same pattern at lower intensity levels. The fluorescence level of the other esophageal layers was barely above background levels. The maximal fluorescence ratio between the squamous basal cell layer and muscularis mucosae was 3 (3 hr after ALA administration). This ratio was 7 between the squamous basal cell layer and submucosa or muscularis (also 3 hr after ALA administration). The results of the p.o. and i.v. groups did not differ significantly.

PBGD and Ferrochelatase Activity

PBGD activity in the mucosa (78.29 ± 10.8 pmol/hr/mg protein) was almost threefold higher than in the muscularis (30.72 ± 7.5 pmol/hr/mg protein, $P < 0.001$; Table 1). The ferrochelatase activity in the esophageal mucosa and muscularis did not differ significantly (0.68 ± 0.08 nmol/hr/mg protein and 0.63 ± 0.09 nmol/hr/mg protein, $P = 0.16$).

Iron Concentration

The amount of iron in mucosa (1.40 ± 0.13 μmol/g dry weight) was lower than that in mus-

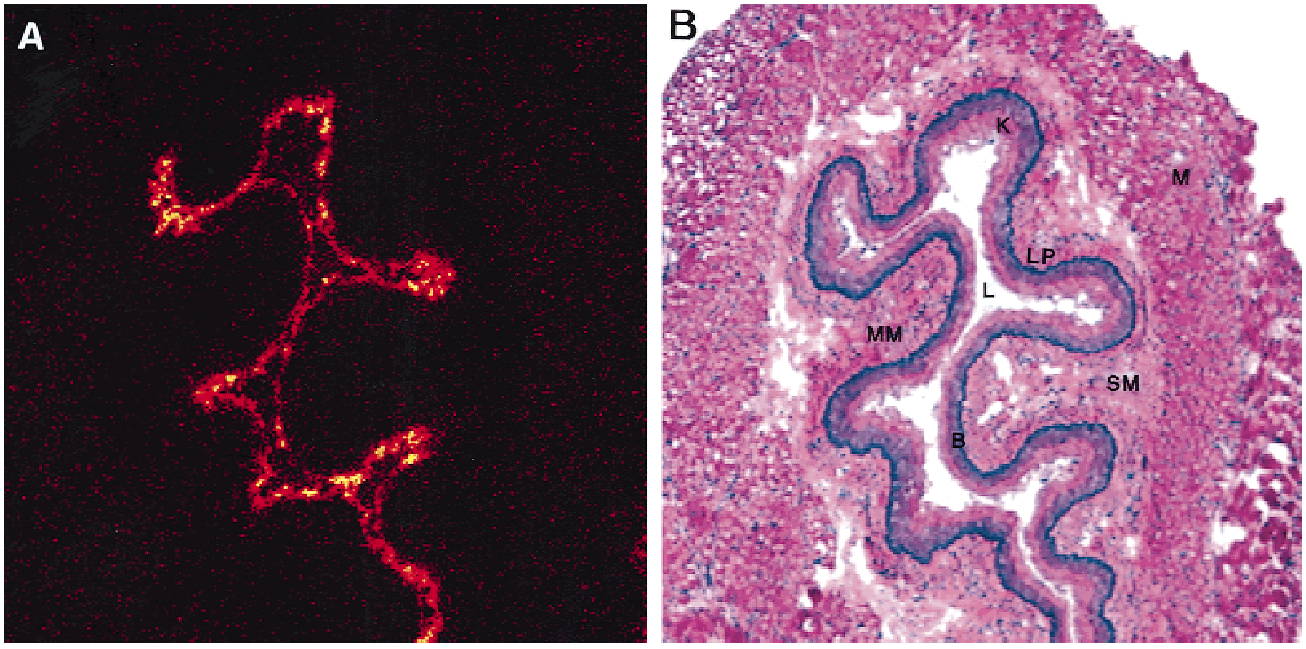

Fig. 2. **A:** Laser scanning microscopic image of a frozen section (10 μm) of a normal rat esophagus 4 hr after receiving intravenous phosphate buffered saline. **B:** Light microscopic image of the same section after staining with hematoxylin and eosin. B, mucosal basal cell layer; K, keratin layer; L, lumen; LP, lamina propria; M, muscularis; MM, muscularis mucosae; SM, submucosa.

cularis (2.15 ± 0.12 μmol/gr dry weight, $P < 0.001$; Table 1).

Barrett's-like Esophagus

Eighteen weeks after an esophagojejunostomy, several histological abnormalities were observed: regenerative thickening, basal cell hyperplasia, keratin cysts, and Barrett's-like columnar metaplasia. The Barrett's-like mucosa had the same histological aspect as that found in man. It consisted of gastric epithelium with glands and foveolae rather than crypts and villi characteristic of jejunal mucosa. The term "Barrett's-like esophagus" is used to emphasize its similarity to human BE. In most animals, the columnar esophageal epithelium is not in continuum with the jejunal epithelium, but it is separated at the site of the anastomosis (marked by the sutures) by a small area lined by squamous epithelium.

Fluorescence Imaging in Barrett's-like Esophagus

Eighteen of the 21 study animals showed Barrett's-like esophagus in the distal segment. In these sections, fluorescence was also restricted to the epithelium of the mucosa but had a heterogeneous aspect (Fig. 5). Some areas showed strong fluorescence, others, especially some deep glands, showed considerably less, and some even no detectable fluorescence. The stromal tissue between the glands showed fluorescence near background level.

Fluorescence intensity did not depend only on the time of illumination after ALA administration but also on the histologic appearance of the columnar mucosa and the quantity of glands in particular. Highest mean fluorescence was found at 3 hr after ALA administration, but the values within each animal and between animals, killed at the same time after ALA administration, differed considerably. Within each animal, we compared the gray values of the columnar mucosa with that of the adjacent squamous epithelium (with regenerative thickening and basal cell hyperplasia, up to 20 cell layers thick, also showing homogeneous fluorescence restricted to the epithelial layer) and the squamous epithelium in the proximal sections (3–5 rows of cells lining the surface). For determination of the gray value of the columnar mucosa, all mucosal glands were included, at some places extending up to the muscularis mucosae and up to 50 rows thick with, besides epithelial cells, stromal cells. At none of the time points studied after ALA administration was the fluorescence of the Barrett's-like epithelium higher than that of the normal mucosa.

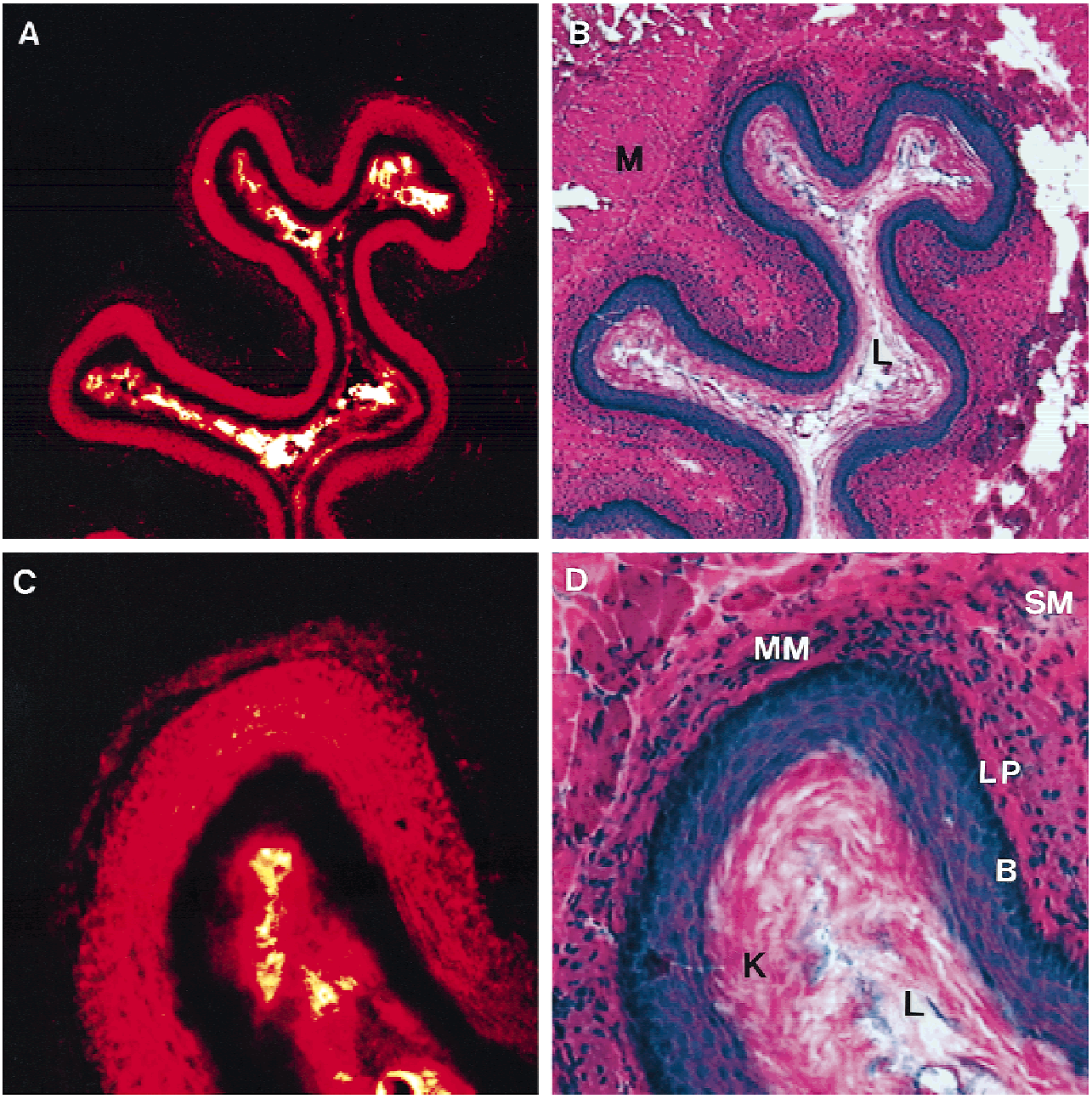

Fig. 3. Fluorescence image of a frozen section (10 μm) of a normal rat esophagus 3 hr after intravenous administration of 5-aminolevulinic acid (200 mg/kg; **A**), together with corresponding section stained with hematoxylin and eosin (H&E; **B**). Fluorescence image (**C**) and corresponding H&E section (**D**) of the same section at higher magnification. B, mucosal basal cell layer; K, keratin layer; L, lumen; LP, lamina propria; M, muscularis; MM, muscularis mucosae; SM, submucosa.

DISCUSSION

This study shows that, in the rat esophagus, ALA-induced porphyrin accumulation occurs selectively in mucosa, whether squamous or Barrett's-like, compared with the muscularis. No selectivity of PpIX accumulation in Barrett's-like epithelium in favor of adjacent squamous epithelium was detected. Maximal porphyrin concentration was found at 3 hr after ALA administration. A possible explanation for the selective accumulation of porphyrins is a higher ratio of PBGD:ferrochelatase in the mucosa compared to the muscularis and a relatively low iron concentration.

ALA-PDT treatment for Barrett's esophagus is, in theory, based on selective ALA-induced porphyrin production in Barrett's mucosa and a much lower production in surrounding normal mucosa [17,27]. Within each animal, we deter-

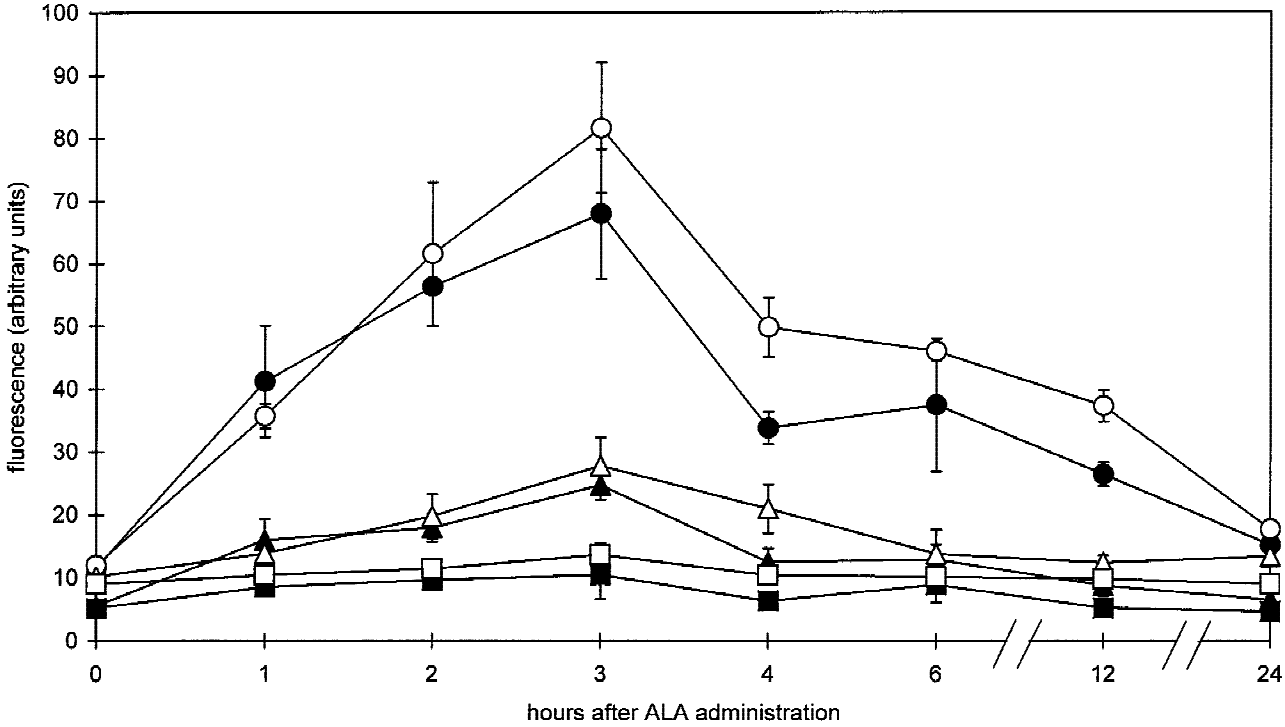

Fig. 4. Gray-scale values of fluorescence ± SEM in tissue layers of the normal esophagus as a function of time after 5-aminolevulinic acid (ALA) administration. Each point indicates the mean level of three rats ± SEM. The levels of control rats are given at 0 hr. Open circles, mucosal basal cell layer, intravenous ALA; solid circles, mucosal basal cell layer, oral gavage ALA; open triangles, muscularis mucosae, intravenous ALA; solid triangles, muscularis mucosae, oral gavage ALA; open squares, rest of the esophageal wall (lamina propria, submucosa, muscularis), intravenous ALA; solid squares, rest of the esophageal wall (lamina propria, submucosa, muscularis), oral gavage ALA.

TABLE 1. Porphobilinogen Deaminase (PBGD), Ferrochelatase Activity, and Iron Concentration in Esophageal Mucosa and Muscularis*

	Mucosa	Muscularis	*P*
PBGD (pmol/hr) of uroporphyrin/mg protein	78.29 ± 10.77	30.72 ± 7.47	<0.001
Ferrochelatase (nmol/hr) of zinc PpIX/mg protein	0.68 ± 0.08	0.63 ± 0.09	0.16
Iron (μmol/g dry tissue)	1.40 ± 0.13	2.15 ± 0.12	<0.001

*Values are expressed as mean ± SEM; n = 10 for all groups.

mined fluorescence in both Barrett's-like and adjacent squamous epithelium at several hours after ALA administration. With respect to its premalignancy, because treatment of BE requires elimination of all the columnar mucosa, we determined the gray value of the Barrett's-like mucosa including the deepest glands and not just of the columnar cells at the surface. No selectivity in porphyrin fluorescence of Barrett's-like versus adjacent normal squamous mucosa was found. Furthermore, the heterogeneity of fluorescence in Barrett's-like epithelium may explain why ALA-PDT for BE in clinical case reports is not always successful and may lead to pseudoregression [16,17].

In the normal esophageal mucosa and muscularis, we quantified porphyrin concentration with the extraction method. Although surgical excision of the mucosa is not at cellular accuracy, the SEMs were relatively small. The results obtained from the quantitative chemical extraction method and laser scanning microscopy (LSM) were supplementary and show that, in normal esophageal mucosa, a considerable amount of porphyrins accumulates. Besides the precise localizing properties, the LSM also provided information on (relative) porphyrin concentrations. Pilot studies (unpublished data) and results of others have indicated that the porphyrin concentration in the normal rat esophagus after administration of 200 mg/kg ALA is high enough to induce excessive photodynamic damage [28].

A possible mechanism for selective ALA-induced porphyrin production is that ALA uptake is higher in tissues that accumulate porphyrins

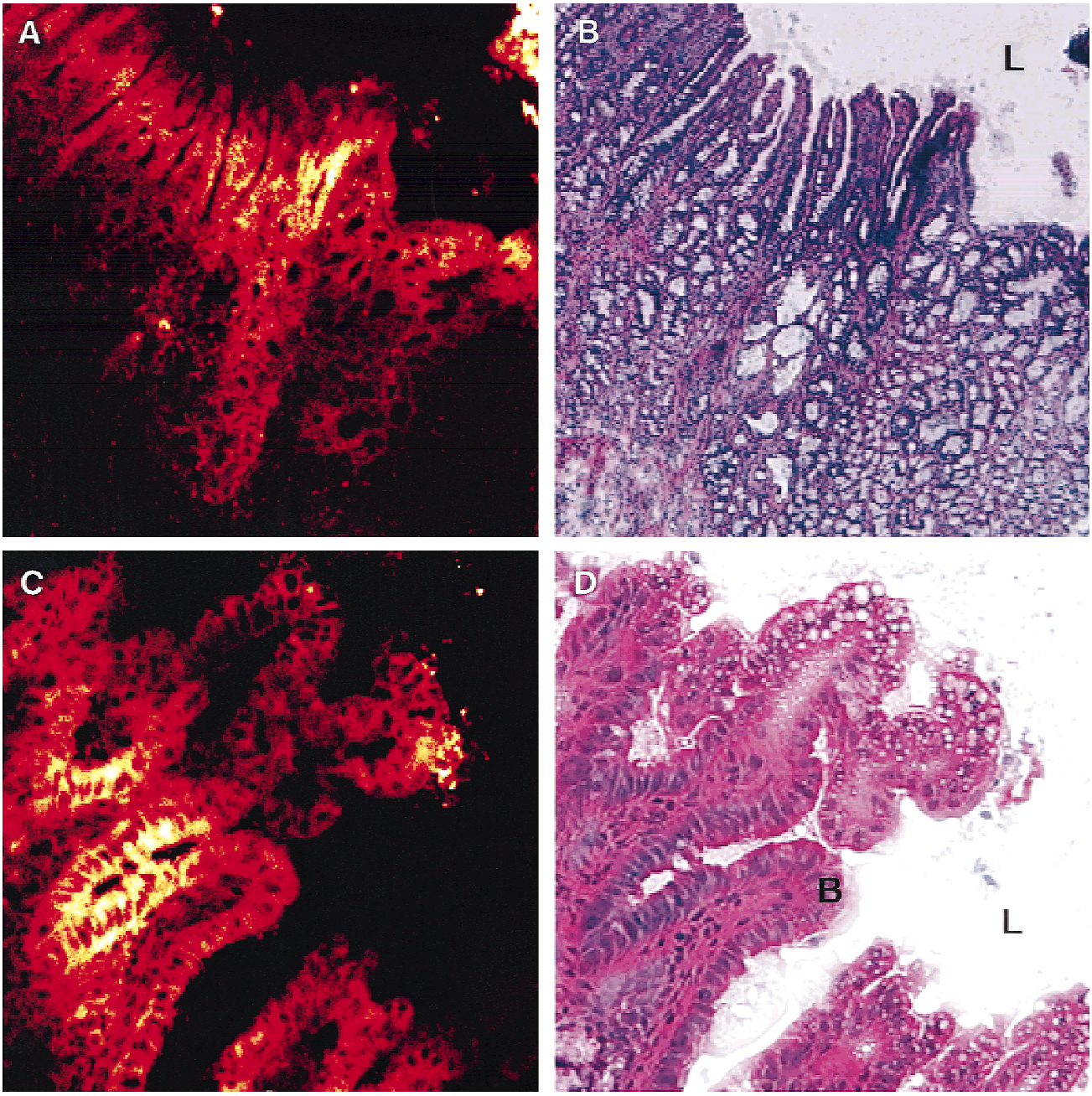

Fig. 5. Fluorescence image (**A**) and a subsequent light microscopic image after staining with hematoxylin and eosin (**B**) of a rat esophagus 3 hr after intravenous administration of 5-aminolevulinic acid and 18 weeks after an esophagojejunostomy, showing columnar epithelium. **C,D:** The same sections at higher magnification. B, Barrett's-like epithelium; L, lumen.

[28,29]. Indeed, ALA concentration in mucosa was higher than that in muscularis (significance was reached only in the p.o. group, $P = 0.03$). No difference in ALA concentration in either mucosa or muscularis was seen between p.o. and i.v. routes of ALA administration. Until detailed information of cellular mechanisms of ALA uptake and efflux is obtained, the reason for ALA to accumulate selectively in specific tissue layers remains unknown.

Another reason for selective porphyrin accumulation in mucosa may be a difference in activity of the porphyrin-forming enzyme PBGD and the porphyrin-converting enzyme ferrochelatase between mucosa and muscularis [11,30,31]. We found no difference in ferrochelatase activity in the mucosa versus the muscularis. However, the PBGD activity was almost threefold higher in the mucosa than in the muscularis. The PBGD:ferrochelatase ratio was higher and thus more advan-

tageous for porphyrin accumulation in mucosa (1.15) than in muscularis (0.49; $P < 0.001$). Previous investigations have shown that a difference in iron concentration could be an explanation for selective porphyrin accumulation in certain cells and that iron chelators could enhance ALA-induced porphyrin accumulation [32,33]. Iron is needed for formation of heme from PpIX. A relatively low iron concentration could be a limiting factor for the formation of heme when high levels of ALA are present, which may result in accumulation of PpIX. Iron concentration in the mucosa was significantly lower than that in muscularis. Whether this relatively low concentration is indeed restricting for heme formation is not clear. Low iron concentration is essential for bacteriostatic and bactericidal properties in blood, lymph, and exudates [34]. For the same reason, iron concentration in epithelial lining, being the first barrier to microorganisms, may be low. However, no other information about iron concentration in epithelial surfaces is available. Iron determination in more tissues and comparison with the level of porphyrin accumulation in these tissues is needed to understand further the role of iron.

Finally, a reason for selectivity of ALA-induced porphyrin accumulation may be a shorter cell-doubling time of tissues that accumulate high porphyrin concentrations [32]. A higher cell turnover implies a higher metabolic activity, especially a higher requirement for heme, an important component in vital respiratory pigment, and therefore a higher production of porphyrins. Although the high fluorescence of the epithelial basal cell layer could be explained by the high turnover of these cells, the fluorescence of the muscularis mucosae cannot. However, a marked similarity between the fluorescence images and corresponding H&E-stained sections was noticed: strong porphyrin fluorescence was associated with intense hematoxylin staining, not only of nuclei, but also of the cytoplasm. Further research is needed to determine the relevance of this observation.

In conclusion, ALA-induced porphyrin accumulation occurs selectively in the normal and Barrett's-like epithelium compared with the muscularis. Therefore, ALA-induced endogenous photosensitization seems highly suitable for PDT treatment of BE. Further clinical studies must be done to determine whether ALA-PDT radically eliminates BE or whether some columnar cells remain because not all cells show porphyrin fluorescence. Probably destruction of all columnar cells requires a second course of ALA-PDT. Moreover, the normal squamous epithelium adjacent to the Barrett's epithelium accumulates porphyrins in a concentration comparable to BE. Therefore, the place of the illumination during treatment is crucial and will determine the semiselectivity of ALA-PDT for BE. Furthermore, oral ALA administration results in the same kinetic pattern of porphyrins as intravenous administration in the rat esophagus. Maximal porphyrin concentration in the mucosa was found at 3 hr; thus, the most efficient PDT effect will be achieved with illumination at 3 hr after ALA administration. However, most selective epithelial damage probably will be attained with treatment at 2 hr after administration because the ratio of porphyrin concentration between mucosa and muscularis is maximal.

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